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(54) Title: PILIN VARIANTS AND USES THEREOF

(57) Abstract

Disclosed are purified polypeptides which, when expressed on the surface of a first cell, enable the first cell to adhere to second cell, the second cell being a human epithelial or endothelial cell, and the polypeptide consisting essentially of the variable region of *Neisseria meningitidis* pilin. Also disclosed are vaccines including these polypeptides, DNA molecules encoding these polypeptides, antibodies which react with these polypeptides, and methods of using these polypeptides, DNAs, antibodies, and vaccines.

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Pilin Variants and Uses Thereof

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1. Field of the Invention

This invention relates to the prevention and treatment of infection by *Neisseria* bacteria. More particularly, this invention relates to polypeptides useful for preparing vaccines and antibodies against *Neisseria* infection, to DNA molecules encoding such polypeptides, and to methods of using such polypeptides, vaccines, DNAs, and antibodies.

2. Background of the Invention

The *Neisseria* is a genus of bacteria that includes two gram-negative species of pyogenic cocci pathogenic for humans: *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *N. meningitidis* is a major cause of bacterial meningitis in humans, especially children. The disease characteristically proceeds from asymptomatic carriage of the bacterium in the nasopharynx to invasion of the bloodstream and cerebro-spinal fluid in susceptible individuals.

N. meningitidis has a polysaccharide capsule whose diversity of component antigenic polysaccharide molecules has resulted in the classification of ten different serogroups. Of these, group A strains are the classic epidemic strains; group B and C are generally endemic strains, but C occasionally causes an epidemic outbreak. All known group A strains have the same protein antigens on their outer membranes, while group B strains have a dozen serotypes or groupings based on the presence of principal outer membrane protein antigens (as opposed to polysaccharides).

The adhesion of *N. meningitidis* to human mucosal epithelial cells is of primary importance in the pathogenicity of this bacterium (DeVoe, 1982, Microbiol. Rev. 46: 162-190). Pili, or filamentous surface protein structures mainly composed of repeating 18 to 24 kilodalton subunits called pilin, have been

shown to play an essential role in the colonization step of these bacteria (Heckels, 1989, Microbiol. Rev. 2: S66-S73; Stephens *et al.*, 1984, Infect. Immun. 46: 507-513; Virji *et al.*, 1991, Mol. Microbiol. 5: 1831-1841). However, among piliated *N. meningitidis* strains, both inter- and intrastrain variability exist with respect to their degree of adhesion to epithelial cells *in vitro* (Virji *et al.*, 1982, Mol. Microbiol. 6: 1271-1279). This suggests that factors other than the presence of pili *per se* are involved in this process.

N. gonorrhoeae is the cause of the well known, sexually transmitted disease, gonorrhea, which produces acute suppuration of the mucous membranes of the genito-urinary tract and of the eye followed by chronic inflammation and fibrosis. This specie of *Neisseria* lacks a true polysaccharide capsule, but, like *N. meningitidis*, possess pili that are important in mediating its attachment to certain types of epithelial cells. *N. gonorrhoeae* have been classified into at least sixteen distinct serotypes, each of which has characteristic antigenic determinants associated with the pili, a fact which renders both diagnosis and immunization difficult. The infectivity of the organism is extremely high, and it has been estimated that a single sexual encounter with an infected partner results in a 20-30% probability of acquiring the disease. If left untreated, relapses are to be expected, as resistance to re-infection does not appear to develop.

The course of the disease involves colonization of the mucous membranes by the bacterium, a process which is mediated by the attachment of the colonizing cell to the surface membrane by means of the pili associated with its cell wall. After attachment, the gonococcus passes through the epithelium to the epithelial surface where it can be blocked by anti-pilus antibody. However, the use of pilus immunogens of such antibodies as vaccines has been rendered impractical by the lack of cross reactivity among strains.

Historically, infections of both *N. meningitidis* and *N. gonorrhoeae* were treated chemoprophylactically with sulfonimide drugs. However, with the development of sulfonamide-resistant strains came the necessity of using alternative modes of therapy such as antibiotic treatment. More recently, the drug treatment of choice includes the administration of high grade penicillin.

However, the success of antimicrobial treatment is decreased if therapy is not initiated early after infection.

Gonococcal infection has also been treated with penicillin, ampicillin, or amoxicillin, tetracycline hydrochloride, and spectinomycin. Unfortunately, because the incidence of infections due to penicillinase-producing bacteria has increased, several new, more expensive ß-lactam antibiotics have been used in treatment. Despite the fact that existing antibiotics have decreased the serious consequences of gonorrhea, their use has not lowered the incidence of the infection in the general population.

Prevention of meningococcal disease has been attempted by chemoprophylaxis and immunoprophylaxis. At present, rifampin and minocycline are used, but only for humans in close contact with an infected person as this treatment has a number of disadvantages. The only commercially available vaccine against meningococcal meningitis has as its major component the bacterial polysaccharide capsule. In adults this vaccine protects against serogroups A, C, Y and W135. It is not effective against serogroup B, and is ineffective in children against serogroup C. Thus far, immunoprophylaxic preventive treatment has not been available for *N. gonorrhoeae*.

Thus, what is needed are better preventative therapies for meningococcal meningitis and gonorrhoeae including more effective, longer lasting vaccines which protect across all of the serogroups of *N. meningitidis* and all the serotypes of *N. gonorrhoeae*. In addition, better methods are need to treat meningococcal and gonococcal infection.

SUMMARY OF THE INVENTION

It has been discovered that clones of *Neisseria* displaying higher than normal adhesiveness to epithelial cells have variant forms of the pilin protein. Furthermore, it has been determined that a portion of this protein, namely its variable region, confers adhesiveness to piliated bacteria and hence plays a role in infectivity. These discoveries have been exploited to develop the present

invention, which concerns the utilization of a portion of the pilin protein, directly or indirectly, to treat and prevent *Neisseria* infection.

In one aspect, the invention relates to a purified polypeptide which, when expressed on the surface of a first cell, enables that cell to adhere to a second cell. The second cell is a human epithelial or endothelial cell. The polypeptide consists essentially of the variable region of *N. meningitidis* pilin. In one embodiment of the invention, the first cell is a bacterial cell such as an *N. meningitidis* or *N. gonorrhoeae* clone that is highly adhesive. The first cell may also be a prokaryotic or eucaryotic cell that has been genetically engineered to express the pilin variable region polypeptide on its surface.

The term "variable region" as used herein refers to the region of the *N. meningitidis* pilin protein about 50 residues downstream from the amino terminal conserved or constant region to its carboxy terminus. The amino acid sequence and length of this variable region varies from protein variant to protein variant, from clone to clone (of a single species), and from species to species. The term "pilin variable region polypeptide" is meant to encompass the variable region of a native pilin protein as well as genetically engineered or biochemically synthesized polypeptides having an amino acid sequence sufficiently duplicative of the amino acid sequence of a native pilin variable region such that the analog has the biological activity and immunogenicity of a native pilin variable region. In preferred embodiments of the invention, the amino acid sequence of this polypeptide comprises the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or 13.

In other embodiments, the polypeptide includes specific portions of the variable region of the pilin protein such as the hypervariable regions. In some embodiments, this polypeptide also includes regions within the variable region that are less variable which flank the hypervariable regions selected to make up the polypeptide.

The polypeptides of the invention also take the form of a therapeutic formulation which includes a physiologically acceptable carrier. This formulation is used in methods of preventing *Neisseria* infection in a mammal. In this

method, the formulation is administered to a mucosal membrane of the mammal, such as one found in the nose, esophagus, cervix, or urinary tract, in an amount sufficient to prevent the binding of *Neisseria* to epithelial cells in the membrane. The polypeptide in the formulation binds to the epithelial cells of the mucosal membrane to the exclusion of *Neisseria*, thereby preventing its infection.

The polypeptides of the invention are also provided in the form of a vaccine protective against *Neisseria* infection in a mammal. In one aspect of the invention, the vaccine is used in a method of preventing *Neisseria* infection wherein the vaccine is administered to the mammal in an amount sufficient to elicit the production of antibodies in the mammal that react with pilin.

In another aspect of the invention the pilin variable region polypeptides are used to prepare antibodies with which they react. Preferred antibodies are monoclonal antibodies. These antibodies are used in other embodiments of the invention including therapeutic formulations and methods of treating a mammal infected with a piliated bacteria from the *Neisseria* genus. In such methods, the antibody, along with a physiologically acceptable carrier, is administered to the mammal in an amount sufficient to enable the antibody to bind to all available pili of the infecting bacteria, thereby inhibiting the adhesion of the bacteria to a mammalian cell, and hence thwarting further infection.

The antibodies of the invention are also used in methods of preventing the adhesion of a bacteria of the *Neisseria* genus to a human epithelial cell. In these methods, an epithelial or endothelial cell is treated with the antibody in an amount sufficient to hinder the ability of pilin in the pili of these bacteria to adhere to these human cells.

Further, the invention provides an isolated DNA encoding the pilin variable region polypeptide. Other DNAs of the invention encode specific portions of the pilin variable region gene that are required for antigenicity and adhesion. In some aspects of the invention, this DNA encodes the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or 13.

This DNA is utilized to provide other embodiments of the invention, namely, cells transformed with this DNA, methods of targeting a cell-of-interest

to a human epithelial or endothelial cell, and methods of treating a mammal infected with *Neisseria*. In the targeting method, the DNA is used to transform a cell-of-interest, such as a prokaryotic or eucaryotic cell, which is then cultured to express the pilin variable region on its surface. The targeted epithelial or endothelial cell is then contacted with the transformed cell for a time sufficient to allow the pilin variable region polypeptide to adhere to the epithelial cell.

In the treatment method, the DNA of the invention is provided with a carrier in the form of a therapeutic formulation. This formulation is administered to the mammal such that the DNA is expressed in the mammal as a pilin variable region polypeptide in an amount sufficient to elicit the production of antibodies reactive with the polypeptide. In preferred forms of the invention, the carrier includes a cell which expresses the DNA such as a cell which normally expresses the DNA or a cell transformed with, and capable of expressing, the DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

Fig. 1 is a graphic representation of the adhesion of derivatives of nonpiliated *N. meningitidis* strain 8013 cells onto Hec1B cells;

Fig. 2 is a Western blot of the outer membrane proteins of clones 1 and 2 using rabbit polyclonal antiserum raised again whole proteins of the high adhesive clone 2. Part A, nonabsorbed serum; part B, serum absorbed against whole bacteria from the low-adhesive clone 1; part C, serum absorbed against whole bacteria from the low adhesive clone 1. Lane 1: outer membrane proteins of a P- derivative of clone 2; lane 2: outer membrane proteins of the high-adhesive clone 2; and lane 3: outer membrane proteins of the low-adhesive clone 1;

Fig. 3A is a schematic of representation of the 683 bp *Hin*dIII-*Cla*I fragment encoding the *pil*E gene of class I *N. meningitidis* strains according to

Potts and Saunders (1988, Mol. Microbiol. 2: 647-653). The arrows indicate the location of /some of the primers used in experimentation;

Figure 3B is a schematic representation of the deduced amino acid sequence of the variable region of the low- and high-adhesive polypeptide derivatives starting at residue 54 of the mature pilin protein. Four different sequences are listed: SA; SB; SC; and SB*. Asterisks (*) indicate sequence identity with SB, and dashes (-) represent gaps introduced for alignment purposes;

Figure 4A is a schematic representation of the *pilE*::Km fusion construction. The arrows indicate the location of the primers used in this experiment. The solid box indicates the location of the *Neisseria* DNA uptake sequence; and

Figure 4B is a graphic representation of the adhesion of the transformants (expressing a defined pilin variant gene) to Hec1B cells. These transformants are designated by the name of the strain followed by the name of the pilin sequence (in parenthesis) carried on the pilin-kanamycin fusion.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention provides pilin variable region polypeptides which confer adhesiveness to the cells on which these polypeptides are expressed, and hence, which enhance the ability of these cells to infect human tissue.

Expression of bacterial virulence factors has been shown to be under the control of systems which are coordinately regulated and responsive to various environmental signals (Miller *et al.*, 1989, Science 243: 916-922). In the case of antigenic variation (which, broadly defined, to the ability of a bacteria to alter the antigenic character of its surface components), the selective pressure exerted by the host could, by selecting certain variants, direct the pathogenicity of the bacterium. Antigenic variation has been considered a means by which virulent bacteria evade the host immune system. Recently, antigenic variation of the cytoadherence protein of *Plasmodium falciparum* was correlated with variations in cytoadherence (Roberts *et al.*, 1992, Nature 357: 689-692). In *N. gonorrhoeae*, epithelial cell invasion has been correlated with the expression of

certain capacity (*opa*) variants (Makino *et al.*, 1991, EMBO. J. 10: 1307-1315; Weel *et al.*, 1991, J. Exp. Med. 173: 1395-1405).

The data obtained from the experiments described and set forth below in the Examples provides genetic evidence that bacterial pathogens can use antigenic variation to modulate the expression of a virulence factor such as pilin.

It has been discovered that some infective clones of *N. meningitidis* have variant forms of the pilin protein that are highly adhesive. To determine that the source of this increased adhesivity is due, at least in part, to the presence of antigenic variations of the pilin variable region, piliated derivatives of an *N. meningitidis* strain were examined.

Piliated (P+) revertants were obtained from a nonpiliated (P-) derivative of the *N. meningitidis* clinical isolate 8013 belonging to serogroup C [Nassif *et al.*, Nature (submitted)]. Clones 1 and 2 were isolated after one passage of 8013 onto Hec1B cells, a cell line derived from a human endometrial adenocarcinoma. Clones 3 and 4 are spontaneous P+ revertants isolated by growth of 8013P- on agar plates. Piliation was established by electron microscopy of negatively stained bacteria. All four P+ derivatives display a similar degree of piliation.

The adhesiveness of several of these P+ revertants is shown in Figure 1. The value reported for each strain represents the mean value and standard error of at least 5 independent adhesion assays. Strains 1, 4, 3L1 and 3L2 were considered low-adhesive. Clones 2 and 3 were considered high-adhesive. The adhesion of the original 8013 nonpiliated (P-) strain is not represented and was less than 0.01.

A great variation in the adhesion phenotype exists among these clones. For example, clones 2 and 3 are significantly more adhesive than clones 1 and 4. These results indicate that acquisition of the adhesive phenotype is not dependent upon *N. meningitidis* contact with epithelial cells.

In order to determine whether the high-adhesive phenotype was reversible and subsequently, to estimate the frequency with which this variation occurs, adhesion assays were performed on 240 individual colonies derived from the high-adhesive clone 3. Two such colonies, 3L1 and 3L2, were non-adhesive (Figure

1). 3L1 was P-. On the other hand, 3L2 had the same amount of pili as the parental clone 3, confirming that factors other than piliation are involved in the adhesion of *N. meningitidis* to human cells.

To identify the *N. meningitidis* component(s) responsible for this difference in adhesion, a rabbit polyclonal antiserum was raised against total proteins of the high-adhesive clone 2. This antiserum was then diluted to 1/4000 and absorbed against the outer membrane proteins of: 1) a P- derivative of clone 2; the high adhesive clone 2; and the low adhesive clone 1. The P- derivative of clone 2 was constructed by insertion of a kanamycin gene in the constant region of the *pil*E locus or clone 2, and produces no pilin.

The results are shown in Figure 2. The serum absorbed against the low-adhesive clone 1 recognizes an 18.5 kilodalton protein of the high-adhesive clone 2 (part C, lane 2). The absence of a similar band in part the P- derivative (part C, lane 1) suggests that the 18.5 kilodalton band in lane 2, part C is pilin. These results show that the absorbed serum still reacts strongly with the pilin of the high-adhesive derivative, and suggest that the low-adhesive clone 1 produces a pilin which is antigenically different from that of the high-adhesive clone 2.

N. meningitidis pilin undergoes extensive antigenic variation (Olafson *et al.*, 1985, Infect. Immun. 48: 336-342), which has resulted in the development of two classes of pili: class I is similar to the gonococcal pilus and reacts with the SM1 monoclonal antibody; class II pili do not bind SM1 and are unrelated to *N. gonorrhoeae* pili (Perry *et al.*, 1988, J. Bacteriol. 170: 1691-1697; Virji *et al.* , 1989, J. Gen. Microbiol. xxx: 3239-3251). Like *N. gonorrhoeae*, expression of the pilin gene in *N. meningitidis* occurs at the *pil*E locus. Strain 8013 used in this study is a class I *N. meningitidis* strain and possesses only one copy of the *pil*E locus.

The *Neisseria* pilin gene in the *pil*E locus has a constant (C) region common to all *N. gonorrhoeae* pilins, and class I *N. meningitidis* pilins, and a variable (V) region. These regions are shown in Figure 3A. The C region encodes approximately the first 50 residues of each mature pilin and is

substantially invariant in sequence. The V region encodes the C-terminal 107 residues of mature pilin. Variation of the nucleotide sequence and length within this region is responsible for pilin antigenic variation (Gibbs *et al.*, 1989, Nature 338: 651-652; Seifert *et al.*, 1988, Microbiol. Rev. 52: 327-336; Seifert *et al.*, 1988, Nature 336: 392-395). This variable region consists of several hypervariable regions interspersed within a number of less variable, more conserved regions of the protein. The hypervariable regions are characterized by insertions and deletions of one or more codons in multiple sites, as well as single codon changes. Epitope mapping studies with pilin-specific monoclonal antibodies indicate that this region encodes the most antigenic portion of pilin (Nicholson *et al.*, 1987, J. Gen. Microbiol. 133: 825-833; Seifert *et al.*, 1988, Microbiol. Rev. 52: 327-336).

To determine whether a correlation exists between the degree of adhesion and the presence of a particular pilin variant, the nucleotide sequence of the *pilE* variable region of the different isolates (SA, SB, SC, and SB*) was determined, and the corresponding amino acid sequence deduced.

The results are shown in Figure 3B. Both low-adhesive P+ derivatives (clones 1 and 4) yielded the same sequence, which is termed "SA" (SEQ ID NO: 11). The same DNA sequence was found in the parental strain, 8013P-. On the other hand, both high-adhesive isolates (clones 2 and 3) expressed a completely different pilin gene, termed "SB" (SEQ ID NO:12). Since these clones were isolated independently, these data indicate that the high-adhesive phenotype is correlated with the production of a specific pilin variant. This is corroborated by the fact that 3L2, the spontaneous low-adhesive P+ derivative of clone 3, expresses a pilin sequence different from SB, here termed "SC" (SEQ ID NO:14).

To definitively establish the role of pilin antigenic variation as a regulator of *Neisseria* adhesion to Hec1B cells, pilin sequences were exchanged between low- and high-adhesive isolates. The *pilE* genes SA and SB from low-adhesive clone 4 and high-adhesive clone 3, respectively, were cloned into *E. coli*, and a kanamycin (Km) resistance gene was transcriptionally fused to the 3' end of each. Figure 4A is a schematic representation of this fusion. Each SA::Km and

SB::Km fusion was initially shuttled by transformation into the *N. meningitidis* strain from which its *pil*E component was originally isolated, i.e., clones 4 and 3, respectively.

Figure 4B compares the adhesion of the transformants (expressing a defined pilin variant gene, to Hec1B cells. Hec1B is a human endometrial adenocarcinoma cell line obtained from the American Type Culture Collection. The values represent the mean and standard error calculated from at least three experiments. The resultant Km^r transformants displayed the same adhesive phenotype as the parental clones containing a pilin sequence without kanamycin fusion. The sequence of the *pil*E locus in these transformants indicated that the pilin gene in the transformant of clone 3 differed slightly from SB. These modifications are presumably the result of secondary recombination event. They are located far upstream from the site at which differences were observed between SA and SB (Figure 3B). This new variant was termed SB* (SEQ ID NO:13). Its amino acid is compared to those of SA, SB, and SC in Figure 3B.

Since clone 3 expressing the SB*::Km fusion was highly adhesive, DNA from this strain was used to transform the low-adhesive clone to 4 to Km resistance. This exchange of pilin alleles converted the phenotype of clone 4 from low-adhesive to high-adhesive (Figure 4B). Similarly, the introduction of SA::Km into the high-adhesive clone 3 produced a low-adhesive variant (Figure 4B).

Taken together, the results show that adhesion of *Neisseria* to HecIB cells requires the expression of sequences contained in SB or SB*, and that the adhesiveness of *Neisseria* can be modulated by pilin antigenic variation.

Considering the limited amount of *N. meningitidis* sequence flanking the antibiotic resistance gene, SA::Km and SB::Km were first shuttled by transformation from *E. coli* into *N. meningitidis* clones 4 and 3, respectively, to generate the transformants 4 (SA) and 3 (SB*). Transformation into *N. meningitidis* was performed as described by Seifert *et al.* (1988, Nature 336: 392-395). Transformants were selected on GCB agar containing kanamycin (100 μg/ml). Since the Km gene is not under the control of its own promoter, Km^R

transformants could only arise by recombination with the *pil*E locus, and not with a silent locus. This was confirmed by Southern blotting of *Pvu*II and *Cla*I digested chromosomal DNAs of the transformants and probing with (i) the kanamycin gene, and (ii) a fragment encoding the constant region of the *pil*E gene. This latter sequence was obtained by amplification between primer 1 (SEQ ID NO:1) (see Figure 3) and primer 9 (5'-GCC GCT ACA GAG TAT TAC CTG-3') (SEQ ID NO:9).

A Western blot using outer membrane proteins of the transformants probed with the monoconal antibody SM1 (Virji *et al.*, 1983, J. Gen. Microbiol. 129: 2761-2768) verified that the transformants were producing pilin. Furthermore, electron microscopy confirmed that the transformants were P+. Sequencing of the variable region of the *pil*E gene of the transformants was performed as described for Figure 3. The transformant of clone 3 was expressing a pilin variant slightly different from SB. This new variant sequence was named SB* and the strain, 3(SB*), was highly adhesive.

In a second set of transformations the SB*::Km fusion of 3(SB*) was transformed into clone 4, and the SA::Km fusion of 4(SA) was introduced into clone 3, yielding transformants 4(SB*) and 3(SA), respectively. To avoid the cotransfer of the Km resistance gene and sequences located beyond the 120-bp fragment present at the 3' end of the *pil*E locus, meningococcal DNA was first digested overnight with *Cla*I (Figure 3A). The presence of pili and the correct insertion of the KM resistance gene in these transformants were confirmed as described above. The sequence of the variable region of the *pil*E locus for each transformant was identical to that of the transforming DNA.

The pilin variable region polypeptides of the invention may take the form of a therapeutic formulation which includes a physiologically acceptable carrier. This formulation is useful in methods of preventing *Neisseria* infection in a mammal. In this method, the formulation is administered to a mucosal membrane of the mammal, such as one found in the nose, esophagus, cervix, or urinary tract, in an amount sufficient to prevent the binding of *Neisseria* to epithelial cells in the membrane. Administration may be by topical application of a

pharmaceutical formulation in the form of an aspirated solution, cream, salve, or ointment. The polypeptide in the formulation binds to the epithelial cells of the mucosal membrane to the exclusion of the *Neisseria*, thereby preventing its infection.

The pilin variable region polypeptides of the invention may also be used to produce polyclonal or monoclonal antibodies thereto useful in treating *Neisseria* infection in a mammal and useful in preventing the adhesion of *Neisseria* to a human endothelial or epithelial cell. Polyclonal antibodies can be produced by methods well known in the art. For example, an animal such as a mammal may be inoculated with an immunogen containing the pilin variable region polypeptide and an adjuvant. The polypeptide may be provided to the animal as a whole pilin-bearing *Neisseria* cell, *Neisseria* cell outer membrane, isolated pili, isolated pilin, or isolated pilin variable region. Booster injections may be required to obtain a sufficient antibody titer. Blood or serum is removed from the animal and assayed for the presence of the anti-pilin variable region antibodies by reactivity with the polypeptides of the invention.

Monoclonal antibodies to the variable region polypeptides or active fragments of such antibodies can be generated by applying generally known cell fusion techniques (*see, for example*, Kohler and Milstein, 1976, Eur. J. Immunol. 6: 511-519; Schulman *et al.*, 1978, Nature 276: 269-270) to obtain a hybridoma producing the antibody. Although somatic call hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed, e.g., by viral or oncogenic transformation of B lymphocytes. The monoclonal antibody so generated may be subjected to proteolysis to obtain the active fragment such as Fv, Fab, or $(Fab')_2$.

For example, monoclonal antibodies may be prepared by obtaining mammalian lymphocytes (preferably spleen cells), committing the lymphocytes to produce antibodies (e.g., by immunizing the mammal with the particular antigenic determinant of interest beforehand), fusing the lymphocytes with myeloma (or other immortal) cells to form hybrid cells, and then culturing a selected hybrid

cell colony *in vivo* or *in vitro* to yield antibodies which are identical in structure and specificity.

In particular, monoclonal antibodies to the pilin variable region polypeptide can be raised by employing whole cells (from a piliated bacterial line such as *N. meningitidis*), outer membrane from such bacteria, pili isolated from such bacteria, purified pilin, or isolated pilin variable region as an antigen. Mice or other animals can be challenged by injection with a solution of such antigen emulsified in complete Freund's adjuvant at weekly intervals. After the initial injection, the booster injections can be administered without adjuvant or emulsified in incomplete Freund's adjuvant. Alternatively, synthetic or biosynthetic pilin variable region polypeptides produced by genetically transfected cells (see discussion below) can be used as immunogens.

Serum samples from the immunized animal can be taken and analyzed by an enzyme linked immunoabsorbent ("ELISA") assay or the like for antibody reaction with the immunization agent. Animals that exhibit polyclonal antibodies titers are sacrificed and their spleens homogenized. Alternatively, the spleen cells can be extracted and the antibody-secreting cells expanded *in vitro* by culturing with a nutrient medium. The spleen cells are then fused with myeloma (or other immortal) cells by the above-referenced procedure of Kohler and Milstein. The hybridomas so produced are screened (i.e., cloned by the limiting dilution procedure of the above-referenced Baker et al. article) to select a cell line producing antibodies which react with *N. meningitidis* pilin variable region polypeptides. Large scale antibody production can be obtained from such anti-pilin variable region-producing cell lines by various techniques, including the induction of ascites tumors (e.g., after priming with pristane) and the purification of such antibodies from the ascites fluid by Protein A-Sepharose affinity chromatography.

For a further description of general hybridoma production methods, see Oi and Herzenberg, 1980, *in* Selected Methods in Cellular Immunology (Mishell & Shiigi, ed.), W.H. Freeman & Co., N.Y.; Scearce and Eisenbarth, 1983, Meth. Enzymol. 103: 459-469; U.S. Patent 4,411,933 issued to Gillis on October

26, 1986, herein incorporated by reference. Human antibodies (i.e., those obtained from human-human or human-animal hybridoma) can be used as well as animal antibodies. For descriptions of human hybridoma production techniques, see U.S. Patent Nos. 4,451,570, 4,529,694, and Zurawski *et al.*, 1980, *in* Monoclonal Antibodies, Plenum Press, New York, also incorporated by reference.

Active fragments such as Fab, $(Fab')_2$, or Fv can be derived from the monoclonal antibodies disclosed herein by a number of techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin and subjected to HPLC gel filtration. The appropriate fraction containing Fab can then be collected and concentrated by membrane filtration or the like. For further description of general techniques for the isolation of active fragments, see for example, Khaw *et al.* (1982, J. Nucl. Med. 23: 1011-1019, incorporated by reference.

The antibodies and fragments used herein can be labeled preferably with radioactive labels, by a variety of techniques other than the above-described Baker et al. technique. For example, the biologically active molecules can also be labeled with a radionucleotide via conjugation with the cyclic anhydride of diethylenetriamine penta-acetic acid (DPTA) or bromoacetyl aminobenzyl ethylamine diamine tetra-acidic acid (BABE). See Hnatowich *et al.* (1983, Science 220: 613-615) and Meares *et al.* (1984, Anal. Biochem. 142: 68-78, both references incorporated by reference) for further description of labeling techniques.

The antibody of the invention may be used to prevent the adhesion of *Neisseria* to a human endothelial or epithelial cells. In this method, the antibody is applied in an amount which saturates the sites on *Neisseria* to which the antibody binds. These same sites are required for binding of the bacteria to the endothelial or epithelial cell, and hence for successful infection of these cells. Saturation may be determined by assaying adhesivity of *Neisseria* applied to the antibody-treated cells.

The antibodies of the invention may be provided in the form of a therapeutic formulation including a physiologically acceptable carrier. Suitable carriers are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the pharmaceutical formulation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or other compounds which enhance the effectiveness of the antibody.

The polypeptides of the invention may be used to prepare vaccines. Preparation of vaccines which contain polypeptide sequences as active ingredients is well understood in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions. However, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1 to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of manitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25 to 70%.

The polypeptide may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts, include the acid additional salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges are of the order of several hundred micrograms active ingredient per individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in one or two week intervals by a subsequent injection or other administration.

DNA molecules encoding the polypeptides of the invention may be isolated and prepared from *Neisseria* using standard molecular biology methods (see, e.g, Sambrook *et al.* (1990, Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y.). Alternatively, the DNA may be prepared synthetically using an automated DNA synthesizer once its desired sequence is known. The sequence can be determined by sequencing the DNA obtained from pilin-expressing cell.

The DNA so prepared may be used to target a cell-of-interest to another cell having receptors for pilin-bearing cells such as endothelial and epithelial cells. A cell-of-interest may be an effector cell, such as a lymphocyte, killer cell, or other cell having desirable characteristics. It must also be able to express the

DNA when it is transformed therewith. The cell-of-interest is transformed with the DNA and cultured so that it expresses the pilin variable region polypeptide on its surface, enabling it to adhere to a targeted epithelial or endothelial cell.

The DNA of the invention may also be used in the form of a therapeutic formulation for treating *Neisseria* infection in a mammal. The therapeutic formulation includes a physiological carrier such as one described above which is not be detrimental to the structure or functional characteristics of the DNA. This therapeutic formulation is administered to the mammal. Administration may be via conventional parenteral injection, for example, either subcutaneously or intramuscularly. Alternatively, the therapeutic formulation may be administered as a suppository or topical solution. Once administered to the mammal, the DNA is expressed as pilin variable region polypeptide. This occurs when the DNA so administered transforms a cell in the mammal and is expressed by that cell. The polypeptide so produced must be present in such an amount that it elicits an immune response within the mammal, including the production of antibodies which react with the pilin variable region polypeptides.

Various animal models can be used to study the efficacy of the polypeptide formulations, vaccines and antibodies of the invention. Such models include monkeys, rabbits, guinea-pigs, rats, mice, and chicken embryos. The mouse and infant rat intraperitoneal (i.p.) infection models are useful in this regard. Another particularly appropriate animal model utilizes infant rats and mice who have been exposed to *Neisseria* via intranasal (i.n.) instillation (Mackinnon *et al.*, 1992, Microbial Pathol. 12: 415-420).

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLE 1

Cell Preparation

Hec1B cells were maintained in DMEM supplemented with 10% fetal calf serum and incubated at 37°C under 5% CO_2. The day before infection, confluent

monolayers were trypsinized and seeded into a 24-well tray at a density of 3 x 10^5 cells per well. All adhesion assays were preformed with derivatives of 8013, a *N. meningitidis* serogroup C strain. Clones 1 through 4 are spontaneous P+ revertants of the same 8013 P-. 3L1 and 3L2 were isolated as spontaneous low adhesive derivatives of clone 3. *N. meningitidis* strains were routinely grown on GCB agar containing the supplements described by Kellogg *et al.* (1963, J. Bacteriol. 85: 1274-1279). Each derivative was purified as a single colony and kept in aliquots at about -70°C. In order to minimize secondary variations, all experiments performed throughout this work using these derivatives were done with overnight cultures from the frozen stocks.

EXAMPLE 2

Adhesion Assays

For adhesion assays, bacteria were resuspended in cell culture media at an appropriate density. One ml of this suspension was added to each well. The plates were incubated for 4 hours at 37°C under 5% CO_2. The medium was then removed and the number of CFR present in the supernatant calculated by plating dilutions on GCB agar. Each infected well was then washed 5 times with PBS to remove non-adherent bacteria. The cells were then lifted off the plates by scraping with a dacron swab and resuspended in one ml of media. The number of cell-associated bacteria was then determined by plating. The degree of adhesion was calculated as the ratio of cell-associated CFU/CFU present in the supernatant. The results are shown in Figure 1.

EXAMPLE 3

Preparation of Antisera to *N. meningitidis* Outer Membrane Proteins

N. meningitidis outer membrane proteins were prepared as described in Heckels (1977, J. Gen. Microbiol. 99: 333-341), and separated by SDS-PAGE in a 15% gel. New Zealand White Rabbits were immunized subcutaneously with 10^9 heat-killed bacteria of clone 2. Two boosts were administered at 21 day

intervals. Blood was collected 7 days after the last injection. Absorption of the serum was performed at 37°C using formaldehyde-treated bacteria and heat-killed bacteria. The results are shown in Figure 2.

EXAMPLE 4

Preparation of Pilin Variable Region Genes

To prepare DNA encoding the pilin variable region polypeptides, standard molecular biology techniques were performed according to Sambrook *et al.* (*ibid.*). Briefly, chromosomal DNA was isolated from an overnight culture of the frozen stock according to the method of Nassif *et al.* (1991, J. Bacteriol. 173: 2147-2154). DNA sequences of the *pil*E variable region of each derivative were determined by dideoxy sequencing of PCR amplified products. Amplification was accomplished using primer 1 (5'-CCC TTA TCG AGC TGA TGA TTG-3'), set forth in the sequence listing as SEQ ID NO:1, and primer 2 (5'-CAG CCA AAA CGG ACG ACC CC-3'), set forth in the sequence listing as SEQ ID NO:2.

In order to generate single-stranded DNA, amplified fragments were gel purified and used in another PCR reaction using either primer 3 (5'-GGC AAA TCA CTT ACC GCT TGA-3'), set forth in the sequence listing as SEQ ID NO:3, or primer 4 (5'-GGA AAA TCA CTT ACC GCT TGA-3') set forth in the sequence listing as SEQ ID NO:4. The generate DNA was then sequenced using internal *pil*E primers. The deduced amino acid sequences were aligned using the Pileup Program from the Genetics Computer Group. The results are shown in Figure 3B.

EXAMPLE 5

Preparation of Kanamycin-*pil*E Fused Genes

The transcriptional fusion of the kanamycin resistance gene to *pil*E was engineered in 3 steps using pBluescript (Short *et al.*, 1988, Nucleic Acids Res. 16: 7583-7600). First, the 120 bp fragment located directly after the stop codon of *pil*E was closed after amplification between primer 2 (see Figure 3) and primer

5 (5'-GCC CAA GCT TAT ACC ATA AAT TTT AAA TAA ATG-3') (SEQ ID NO:5). Then, the kanamycin resistance gene, an *aph*-3', was amplified from a recombinant plasmid, pMGC20 (Nassif *et al.*, 1991, J. Bacteriol. 173: 2147-2154), using primer 7 (5'-CGG GAT CCA GAA AAG AGG AAG GAA ATA ATA A-3') (SEQ ID NO:7), and primer 8 (5'-GCT TGC CGT CTG AATGCTTTTTA GAC ATC TAA ATC TAGG-3') (SEQ ID NO:8). The fragment synthesized using these primers contains the open reading frame and the ribosome binding site of the gene but not the promoter sequences (Caillaud *et al.*, 1987, Mol. Gen. Genet. 207: 509-513). This gene was then cloned upstream of the 120 bp fragment described above. Primer 8 also carries the *Neisseria* DNA uptake sequence 5'-GCCGTCTGAA-3' (Goodman *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85: 6982-6986), which is set forth in the Sequence Listing as SEQ ID NO:10. The SA and SB sequences were then cloned upstream of the kanamycin resistance gene after amplification using primer 1 (see Figure 3) and primer 6 (5'CGG GAT CCT TAC CTT AGC TGG CAG ATG AAT c-3') (SEQ ID NO:6).

EXAMPLE 6

Transformation

Transformation into *N. meningitidis* was performed as described by Seifert *et al.* (1988, Nature 336: 392-395). Transformants were selected on GCB agar containing kanamycin (100 μg/ml). Since the Km gene is not under the control of its own promoter, Km^R transformants could only arise by recombination with the *pil*E locus, and not with a silent locus. This was confirmed by Southern blotting of *Pvu*II and *Cla*I digested chromosomal DNAs of the transformants and probing with (i) the kanamycin gene, and (ii) a fragment encoding the constant region of the *pil*E gene. This latter sequence was obtained by amplification between primer 1 (SEQ ID NO:1) (see Figure 3) and primer 9 (5'-GCC GCT ACA GAG TAT TAC CTG-3') (SEQ ID NO:9).

EXAMPLE 7

Confirmation of Adherence-Blocking Ability

To test the ability of antibodies raised against the pilin variable region polypeptides of the invention to block adherence to target cells, an *in vitro* assay is used. An innoculum of piliated *Neisseria* is pre-incubated with varying serum dilutions and then transferred to a chamber containing cultured target cells. Target cells are obtained by growing human epithelial or endometrial monolayers on cover slips. After 30 minutes of incubation, unbound bacteria are removed by repeated washings. The cover slip is strained using Giemsa, and the number of adhering bacteria counted.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: State of Oregon
(B) STREET: Oregon Health Sciences Univ., 3181 S.W. Sam Jackson Park Road
(C) CITY: Portland
(D) STATE: Oregon
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 97201-3098
(G) TELEPHONE: 503-494-8200
(H) TELEFAX: (503)-494-4729

(ii) TITLE OF INVENTION: Pilin Variants and Uses Thereof

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/US93/

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /number= 1 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCTTATCGA GCTGATGATT G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..10
(D) OTHER INFORMATION: /number= 2 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGCCAAAAC GGACGACCCC 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /number= 3 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCCGTCACAG AGTATTACCT G 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /number= 4 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGAAAATCAC TTACCGCTTG A 21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /number= 5 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCCAAGCTT ATACCATAAA TTTTAAATAA ATG 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..31
(D) OTHER INFORMATION: /number= 6 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGGATCCTT ACCTTAGCTG GCAGATGAAT C 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..31
(D) OTHER INFORMATION: /number= 7 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGGATCCAG AAAAGAGGAA GGAAATAATA A 31

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..39
(D) OTHER INFORMATION: /number= 8 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCTTGCCGTC TGAATGCTTT TTAGACATCT AAATCTAGG 39

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /number= 9 /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCCGTCACAG AGTATTACCT G 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCGTCTGAA 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Neisseria meningitidis

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: pilE gene
(B) MAP POSITION: amino acid residues 54-163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
His Gly Glu Trp Pro Gly Asp Asn Ser Ser Ala Gly Val Ala Thr Ser
1               5                  10                  15

Ala Asp Ile Lys Gly Lys Tyr Val Lys Glu Val Glu Val Lys Asn Gly
            20                  25                  30

Val Ile Thr Ala Gln Met Ala Ser Ser Asn Val Asn Asn Glu Ile Lys
        35                  40                  45

Gly Lys Lys Leu Ser Leu Trp Ala Lys Arg Gln Asp Gly Ser Val Lys
    50                  55                  60

Trp Phe Cys Gly Leu Pro Val Ala Arg Asp Asp Thr Asp Ser Ala Thr
65                  70                  75                  80

Asp Val Lys Ala Ala Asn Asp Thr Thr Asp Asn Lys Ile Asn Thr Lys
                85                  90                  95
```

His Leu Pro Ser Thr Cys Arg Asp Asp Ser Ser Ala Ser
100 105

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Neisseria meningitidis

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: pilE gene
(B) MAP POSITION: amino acid residues 54-163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Gly Glu Trp Pro Gly Asp Asn Ser Ser Ala Gly Val Ala Thr Ser
1 5 10 15

Ala Asp Ile Lys Gly Lys Tyr Val Gln Ser Val Thr Val Ala Asn Gly
20 25 30

Val Ile Thr Ala Gln Met Ala Ser Ser Asn Val Asn Asn Glu Ile Lys
35 40 45

Ser Lys Lys Leu Ser Leu Trp Ala Lys Arg Gln Asn Gly Ser Val Lys
50 55 60

Trp Phe Cys Gly Gln Pro Val Thr Arg Thr Thr Ala Thr Ala Thr Asp
65 70 75 80

Val Ala Ala Ala Asn Gly Lys Thr Asp Asp Lys Ile Asn Thr Lys His
85 90 95

Leu Pro Ser Thr Cys Arg Asp Asp Ser Ser Ala Ser
100 105

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Neisseria meningitidis

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: pilE gene
(B) MAP POSITION: amino acid residues 54-163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Gly Thr Trp Pro Lys Asn Asn Thr Ser Ala Gly Val Ala Thr Ser
1 5 10 15

Ala Asp Ile Lys Gly Lys Tyr Val Gln Ser Val Thr Val Ala Asn Gly
20 25 30

Val Ile Thr Ala Gln Met Ala Ser Ser Asn Val Asn Asn Glu Ile Lys
35 40 45

Ser Lys Lys Leu Ser Leu Trp Ala Lys Arg Gln Asn Gly Ser Val Lys
50 55 60

Trp Phe Cys Gly Gln Pro Val Thr Arg Thr Thr Ala Thr Ala Thr Asp
65 70 75 80

Val Ala Ala Ala Asn Gly Lys Thr Asp Asp Lys Ile Asn Thr Lys His
85 90 95

Leu Pro Ser Thr Cys Arg Asp Asp Ser Ser Ala Ser
100 105

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Neisseria meningitidis

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: pilE gene
(B) MAP POSITION: amino acid residues 54-163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Gly Glu Thr Pro Gly Asp Asn Ser Ser Ala Gly Val Ala Thr Ser
1 5 10 15

Ala Asp Ile Lys Gly Lys Tyr Val Gln Ser Val Thr Val Ala Asn Gly
20 25 30

Val Ile Thr Ala Gln Met Ala Ser Ser Gly Val Asn Lys Gln Ile Gln
35 40 45

Gly Lys Lys Leu Ser Leu Trp Ala Lys Arg Gln Asp Gly Ser Val Lys
50 55 60

Thr Phe Cys Gly Gln Pro Val Thr Arg Ala Asn Thr Ala Thr Asp Ala
65 70 75 80

Ala Val Thr Ala Ala Ser Asp Thr Thr Ala Asn Lys Ile Asp Thr Lys
85 90 95

His Leu Pro Ser Thr Cys Arg Asp Asp Ser Ser Ala Ser
100 105

What is claimed is:

1. A purified polypeptide which, when expressed on the surface of a first cell, enables the first cell to adhere to second cell, the second cell being a human epithelial or endothelial cell, and the polypeptide consisting essentially of the variable region of *Neisseria meningitidis* pilin.

2. The polypeptide of claim 1 comprising an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12.

3. The polypeptide of claim 1 comprising an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:13.

4. A therapeutic formulation comprising the polypeptide of claim 1 in a physiologically acceptable carrier.

5. The therapeutic formulation of claim 4 wherein the polypeptide comprises the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

6. A method of preventing *Neisseria* infection in a mammal comprising the steps of:

(a) providing the therapeutic formulation of claim 4; and

(b) administering the therapeutic formulation to a mucosal membrane of the mammal in an amount sufficient to prevent the binding of *Neisseria* to epithelial cells in the membrane, the polypeptide in the formulation binding to the epithelial cells of the mucosal membrane to the exclusion of the *Neisseria*, thereby preventing *Neisseria* infection.

7. The method of claim 6, wherein the therapeutic formulation comprises a polypeptide having an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

8. A vaccine protective against *Neisseria* infection in mammals comprising the polypeptide of claim 1.

9. The vaccine of claim 8 wherein the polypeptide comprises an amino acid sequence set forth in the sequence listing as SEQ ID No:12.

10. The vaccine of claim 8 wherein the polypeptide comprises an amino acid sequence set forth in the sequence listing as SEQ ID NO:13.

11. A method of preventing *Neisseria* infection in a mammal, the method comprising the steps of:

(a) providing a vaccine including a purified polypeptide which, when expressed on the surface of a first cell, enables the first cell to adhere to second cell, the second cell being a human epithelial or endothelial cell, and the polypeptide consisting essentially of the variable region of *Neisseria meningitidis* pilin; and

(b) administering the vaccine to the mammal in an amount sufficient to elicit the production in the mammal of antibodies that react with pilin.

12. The method of claim 11, wherein the providing step comprises providing a vaccine that includes a polypeptide having an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

13. An antibody which reacts with the polypeptide of claim 1.

14. The antibody of claim 13 which a monoclonal antibody.

15. The antibody of claim 13 wherein the polypeptide with which the antibody reacts includes an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

16. A therapeutic formulation comprising the antibody of claim 13 in a physiologically acceptable carrier.

17. A method of treating a mammal infected with *Neisseria*, the method comprising the steps of:

(a) providing a therapeutic formulation comprising an antibody reactive with the variable regions of *Neisseria meningitidis* pilin in a physiologically acceptable carrier; and

(b) administering the therapeutic formulation to the mammal in an amount sufficient to enable the antibody to bind to the infecting *Neisseria*, thereby inhibiting the adhesion of the *Neisseria* to a mammalian cell.

18. The method of claim 17, wherein the providing step comprises providing a therapeutic formulation comprising an antibody that reacts with a polypeptide having the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

19. A method of preventing the adhesion of *Neisseria* to a human epithelial or endothelial cell, the method comprising the steps of:

(a) providing the antibody of claim 13; and

(b) treating the epithelial cell with the antibody in an amount which saturates the *Neisseria* binding sites on the epithelial or endothelial cell.

20. The method of claim 19 wherein the antibody reacts with a polypeptide having an amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

21. An isolated DNA comprising the variable region of the pilin gene from *Neisseria meningitidis* and encoding the polypeptide of claim 1.

22. The DNA of claim 21 encoding the amino acid sequence set forth in the sequence listing as SEQ ID NO:12.

23. The DNA of claim 21 encoding the amino acid sequence set forth in the sequence listing as SEQ ID NO:13.

24. A cell transformed with the DNA of claim 21.

25. A method of targeting a cell-of-interest to a human epithelial or endothelial cell, the method comprising the steps of:

(a) providing the DNA of claim 21;

(b) transforming the cell-of-interest with the DNA;

(c) culturing the transformed cell-of-interest such that it expresses the DNA as pilin variable region polypeptide on its surface; and

(d) contacting the targeted epithelial or endothelial cell with the transformed cell for a time sufficient to allow the polypeptide on the surface of the transformed cell to adhere to the epithelial or endothelial cell.

26. The method of claim 25 wherein the providing step comprises providing a DNA encoding the amino acid sequence set forth in the sequence listing as SEQ ID NO:12 or SEQ ID NO:13.

27. A method of treating *Neisseria* infection in a mammal, the method comprising the steps of:

(a) providing a therapeutic formulation comprising the DNA of claim 21 in a carrier; and

(b) administering the therapeutic formulation to the mammal where the DNA in the formulation is expressed as pilin variable region polypeptide in an amount sufficient to elicit the production of antibodies in the mammal which react with the polypeptide.

28. The method of claim 27 wherein the carrier comprises a cell which expresses the DNA.

29. The method of claim 28 wherein the carrier comprises a cell selected from the group consisting of a cell which normally expresses the DNA and a cell transformed with, and capable of expressing, the DNA.

30. The method of claim 27, wherein the DNA encodes a polypeptide having the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:12 or SEQ ID NO:13.

Figure 1

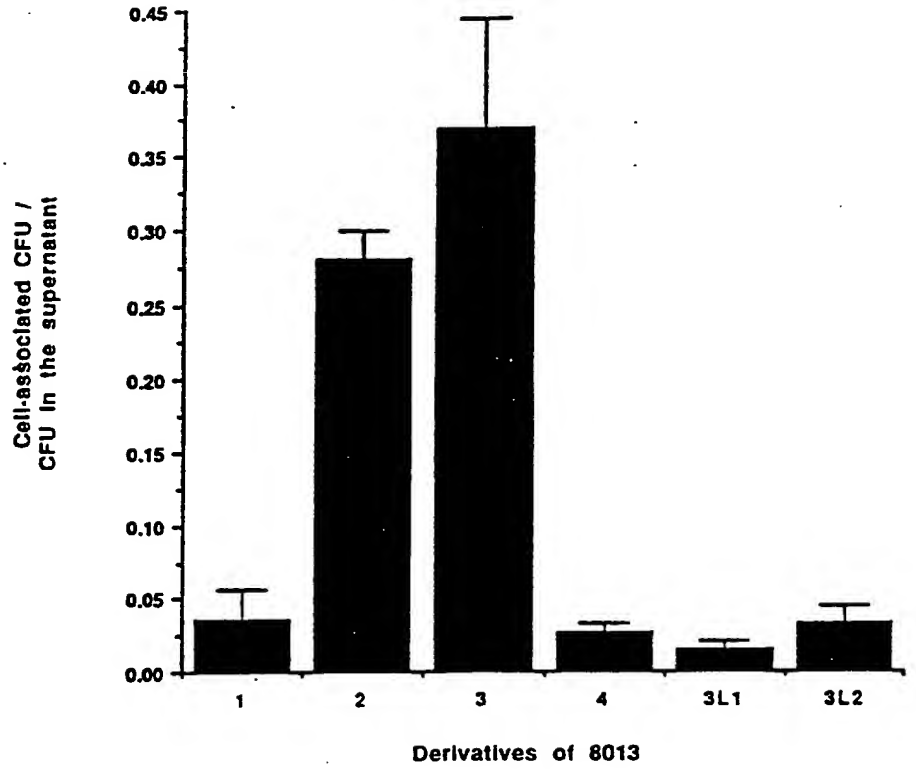

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Cell-associated CFU / CFU in the supernatant
1
2
3
4
3L1
3L2
Derivatives of 8013

Figure 2

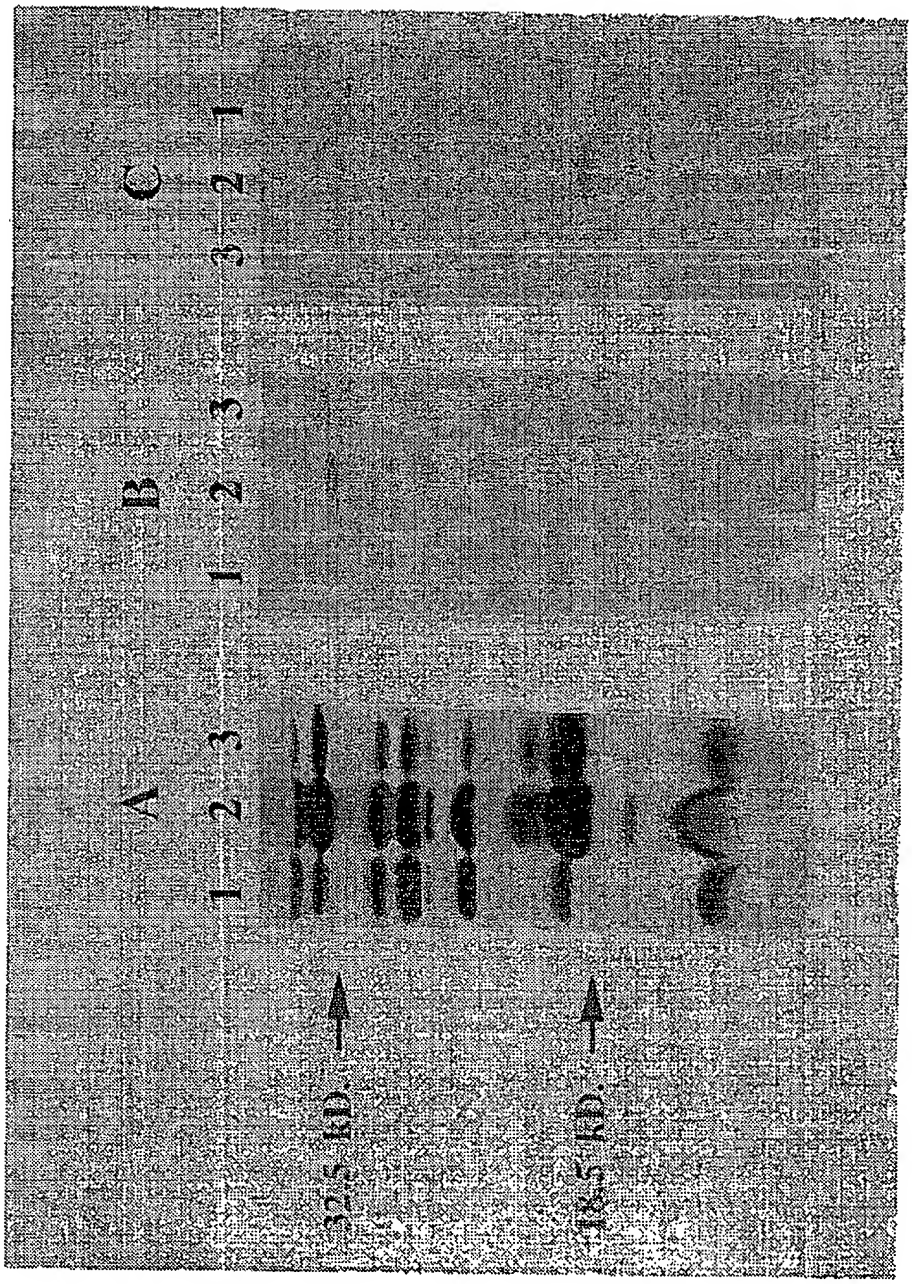


Figure 3A

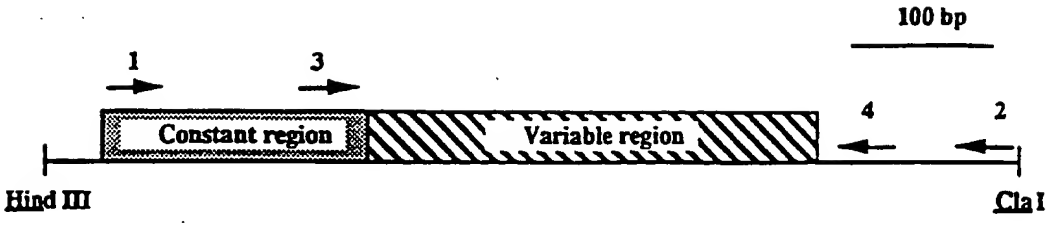


Figure 3B

SA	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SB	His	Gly	Glu	Trp	Pro	Gly	Asp	Asn	Ser	Ser	Ala	Gly	Val	Ala	Thr	Ser	Ala	Asp	Ile	Lys
SC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SB*	*	*	Thr	*	*	Lys	Asn	*	Thr	*	*	*	*	*	*	*	*	*	*	*
SA	*	*	*	*	Lys	Glu	*	Glu	*	Lys	*	*	*	*	*	*	*	*	*	*
SB	Gly	Lys	Tyr	Val	Gln	Ser	Val	Thr	Val	Ala	Asn	Gly	Val	Ile	Thr	Ala	Gln	Met	Ala	Ser
SC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SB*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SA	*	*	*	*	*	*	*	*	Gly	*	*	*	*	*	*	*	*	*	*	Asp
SB	Ser	Asn	Val	Asn	Asn	Glu	Ile	Lys	Ser	Lys	Lys	Leu	Ser	Leu	Trp	Ala	Lys	Arg	Gln	Asn
SC	*	Gly	*	*	Lys	*	*	Gln	Gly	*	*	*	*	*	*	*	*	*	*	Asp
SB*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SA	*	*	*	*	*	*	*	*	Leu	*	*	Ala	*	Asp	Asp	*	Asp	Ser	*	*
SB	Gly	Ser	Val	Lys	Trp	Phe	Cys	Gly	Gln	Pro	Val	Thr	Arg	Thr	-	Thr	Ala	Thr	Ala	Thr
SC	*	*	*	*	*	*	*	*	*	*	*	*	*	Ala	Asn	*	*	*	Asp	Ala
SB*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*	*	*	*	*
SA	*	*	Lys	*	-	-	Asp	Thr	*	*	Asn	-	*	*	*	*	*	*	*	*
SB	Asp	Val	Ala	Ala	Ala	Asn	Gly	Lys	Thr	Asp	Asp	Lys	Ile	Asn	Thr	Lys	His	Leu	Pro	Ser
SC	Ala	*	Thr	*	*	Ser	Asp	Thr	*	Ala	Asn	-	*	Asp	*	*	*	*	*	*
SB*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SA	*	*	*	*	*	*	*	*	*											
SB	Thr	Cys	Arg	Asp	Asp	Ser	Ser	Ala	Ser											
SC	*	*	*	*	*	*	*	*	*											
SB*	*	*	*	*	*	*	*	*	*											

Figure 4A

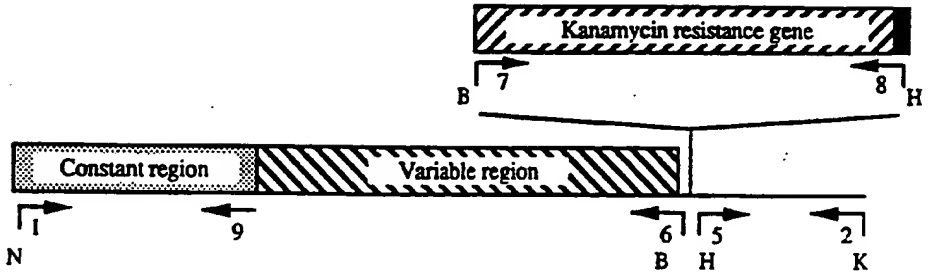
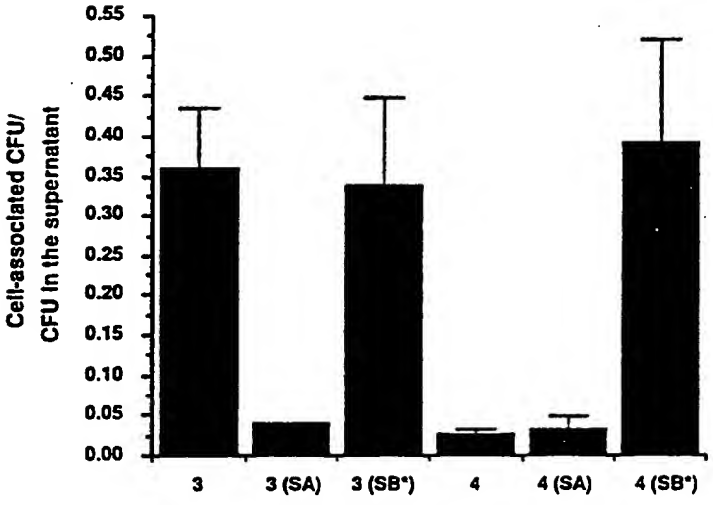

Kanamycin resistance gene
B
7
8
H
Constant region
Variable region
1
N
9
6
B
5
H
2
K

Figure 4B


Cell-associated CFU/
CFU in the supernatant
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
3
3 (SA)
3 (SB*)
4
4 (SA)
4 (SB*)

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 93/09575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/31 C07K14/22 C07K16/12 A61K39/095 A61K39/40 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,85 04654 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 24 October 1985 see page 3, line 9 - line 24 see page 4, line 24 - page 5, line 7 see page 12, line 32 - page 13, line 2 see page 20, line 24 - line 29 --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 1994	01. 03. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR MICROBIOLOGY vol. 2, no. 5 , 1988 pages 647 - 653 W.J. POTTS ET AL. 'Nucleotide sequence of the structural gene for class I pilin from Neisseria meningitidis: homologies with the pilE locus of Neisseria gonorrhoeae' see summary see page 647, right column, paragraph 2; figure 3 see page 649, right column, last paragraph - page 651, right column, paragraph 1	1-3, 21-24
A	WO,A,92 13871 (WASHINGTON UNIVERSITY) 20 August 1992 see page 4, line 1 - page 5, line 20 see page 7, line 27 - page 9, line 5	1-30

1

INTERNATIONAL SEARCH REPORT

:national application No.
PCT/US93/09575

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This inte ational search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 6,7,11,12,17,18,27-30 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/US 93/09575

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8504654	24-10-85	AU-B-	582358	23-03-89
		AU-A-	4159085	01-11-85
		EP-A-	0177583	16-04-86
		JP-T-	61501777	21-08-86
WO-A-9213871	20-08-92	AU-A-	1411492	07-09-92